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SOME HAEMTOLOGICAL PARAMETERS OF INTER-GENERIC HYBRID OF AFRICAN CATFISH (*Clarias anguillaris* x *Heterobranchus bidorsalis*) JUVENILES AND THEIR PURE LINES IN NORTH EASTERN NIGERIA

*** DIYAWARE M.Y.[1]; A.B. HARUNA[2], AND K.A. ABUBAKAR[3]**
[1]Department of Fisheries, University of Maiduguri, Maiduguri, Nigeria
[2]Department of Fisheries, Federal University of Technology, Yola, Nigeria
[3]Department of Biological Science, Federal University of Technology, Yola, Nigeria

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ABSTRACT

Some haematological parameters of inter-generic hybrid African catfish (Clarias anguillaris x Heterobranchus bidorsalis) were investigated to assess the ability of the hybrids to withstand culture conditions in North east Nigeria. The result shows that packed cell volume (PCV), red blood cells (RBC), Haemoglobin (Hb), mean cell haemoglobin concentration (MCHC) and platelets (PLT) were higher in pure line Clarias anguillaris. However, white blood cell (WBC) and lymphocytes (LYM) were higher in female H. bidorsalis x male C. anguillaris (Heteroclarias) hybrid. PCV, MCHC, Hb, and RBC of all the fish species observed in this study were within the healthy ranges for fish. The results of this study show that Heteroclarias have haematological competence for survival in culture conditions in the region.

Keywords: Haematological parameters, inter-generic hybrids, *Clarias anguillaris* x *Heterobranchus bidorsalis.*

INTRODUCTION

In aquaculture, production of hybrid fish plays important role in fast growth, survival, and improvement of genetic traits. Inter-generic hybridization has been performed between African catfish viz: *C. anguillaris* x *H. bidorsalis* (Diyaware *et al*., 2010). Hybridizations between African catfish and Asian catfish; *C. gariepinus* x *C. batrachus* were also carried out Sahoo *et al*., (2003), and *C. gariepinus* × *Heterobranchus longifilis* (Legendre *et al.*, 1992; Nwadukwe 1995), *C. batrachus* x *C. gariepinus* has been attempted by (Rahman *et al*., (1995). Hybridization between *Hepteropneustis fossilis* and *C. gariepinus* have been reported by Muthukumaran and Sukumaran (2005), while that of *C. macrocephalus* and *C. gariepinus* was documented by Na-Nakorn et *al*., (2004). Aluko and Ali (2001) successfully produced eight fast inter-generic hybrid fish from four African catfish species belonging to two genus *Clarias* and *Heterobranchus.*

Haematological indices in these hybrids critical are parameters for the evaluation physiological status and genetic resilience of the fish to resist bacterial infection, fungal and viral disease and withstand intensive culture conditions that compromises immunity. Response to these depends on fish species, age, sexual maturity of fish, and diseases (Luskova, 1997; Golovina and Trombicky, 1989; Golovina, 1996; Zhiteneva *et al*., 1989). Haematological tests and analysis of serum constituents have yielded useful information for detection and diagnosis of metabolic disturbances and disease conditions in fishes (Aldrin *et al*., 1982; Jamalzadeh *et al*., 2009). Like in warm-blooded animals, changes in the blood parameters of fish, occurring from injuries or infections of tissues or organs, can be used to determine extend of the dysfunction or injuries organs or tissues. However in fish, these parameters are more related to the response of the whole organism, i.e. to the effect on fish survival, reproduction and growth than discrete organ or tissue (Vosyliene, 1999).

In recent years, variation in haematological indices were used when clinical diagnosis of fish physiology was required to determine the effects of external stressors and toxic substances due to the close association between the circulatory system and the external environment (Cech *et al*, 1996; Wendelaar and Bonga, 1997). Wepener, (1997) also suggested that haematological and biochemical changes, growth rate and oxygen consumption of fish are used in determining the toxic effects of pollutants.

According to Fernades and Mazon (2003), haematological parameters are closely related to the response of the animal to the environment, an indication that the environment where fish lives could exert some influence on the haematological characteristics (Kori-Siakpere, 1985). Sex of the fish may also influence the blood parameters. Sniezsko, (1960) showed that males consistently had higher packed cell volume values than the females and this has been proposed as means of sexing fish. Blood cell responses are important indicators of changes in the internal and/or external environment of animals (Adeyemo, 2007). A number of haematological indices such as haematocrit (Ht) haemoglobin (Hb), red blood cells (RBCs) are used to assess the functional status of the oxygen carrying capacity of bloodstream and have been used as indicators of the presence of metallic pollutants in aquatic environment (Shah and Altindang 2004).

Red blood count (RBC), concentration of haemoglobin (MCH) and haematocrit (Ht) have been reported to indicate secondary responses of an organism to irritants (Aeyemo, 2007). Decrease in erythrocytes has been reported to be the major and reliable indicators of various sources of stress in fish (Rainza-Paiva *et al.*, 2000; O'neal and Weirich 2001). White blood cells are the main defense cells of the animals. Decrease in these cells indicates vulnerability to stress and infection (Sunmonu and Oyelola, 2008). Drop in red blood cells implies reduction in level of oxygen that is being carried to the tissues and the level of carbon dioxide returned to the lungs will also be reduced. Reduction in mean hemoglobin concentration and mean corpuscular hemoglobin concentration indicates anemia (Sunmonu and Oyelola, 2008). According to myDr (2006), packed cell volume or haemtocrit, red blood cell (RBC), and mean cell haemoglobin concentration (MCHC) are used for diagnosing anaemic condition, while neutrophils and monocytes (differential counts) protects the body against bacterial invasion. Lymphocytes are involved in immune process, producing antibodies against foreign organism, protecting against microbes. Low RBC indicates malnutrition and platelets helps in diagnosing problems associated bleeding or bruising.

Hematological studies in fishes have assumed greater significance because these parameters could be used as an effective and sensitive index to monitor physiological and pathological changes induced by natural or anthropometric factors, such as bacteria and fungi infections or pollution of water environment, pathogenicity of the organism. Haematological study on nutritional effects have been reported Rehulka (2000). Osuigwe *et al.*, (2005) documented some haematological changes in hybrid *Heteroclarias* fed raw and boiled jack beans seed meal. Effects of ascorbic acid on haematological parameters of *C. gariepinus* have been studies *by* (Oluyemi *et al.*, 2008) Rogers *et al*. (2003) concluded that, mechanism of lead toxicity occurs by ion regulatory disruption.

Few authors reported blood profile of Clarrids not exposed to chemicals or feed or disease; *Clarias buthupogon* Kori-Siakpere and Egor (1999), *C. isherensis* Kori-Siakpere, (1985), on hybrid *Heteroclarias* (*H. bidorsalis* and *C. gariepinus*) (Kori-Siakpere *et al*., 2006).

Changes in the blood characteristics of *Clarias gariepinus* caused by stress due to exposure to environmental pollutants, diseases or attack by pathogens have been studied extensively: (Onusiriuka and Ufodike, 2000; Ezeri, 2001; Gabriel, *et al*., 2001; Rehulka 2000; Rehulka, 2002a; Rehulka, 2002b). Maheswaran *et al*., 2008 studied effect of mercuric acid on blood indices of *C.* batrachus, while Okechukwu *et al*., (2007) investigated the haematological indices of *C. gariepinus* to exposure to acute chlorpyrifos-ethyl. Kia-Siakpere *et al*., (2007) reported effects of paraquat on blood indices. These indices have been employed in effective monitoring of the responses of the fish to stressors and thus its health status under adverse conditions.

Information on haemtological profile of Clariids and their hybrids have not been fully documented. Haematological profiles of juvenile hybrids will reveal the possibility of the hybrids to withstand the harsh environmental condition that is likely to be faced by the species during rearing. There is also a need to establish baseline information on haematological profiles of our economically valued fish species for continual assessment of their health status and subsequent diagnosis of disease. The objective of this study is to compare the hematological parameters of hybrid African catfish (*Clarias anguillaris* x *Heterobranchus bidorsalis*) with view to assessing the ability of hybrids to thrive under culture conditions in north east Nigeria.

MATERIALS AND METHODS

Experimental fish

Sixty (60) each of juvenile hybrids of *Clariabranchus* (female *C. anguillaris* x male *H. bidorsalis*), *Heteroclarias* (female *H. bidorsalis* x male *C. anguillaris*), and their pure line *C. anguillaris* x *C. anguillaris* and *H. bidorsalis* x *H. bidorsalis*) were collected from Departmental polyethylene line fish pond and the blood samples collected immediately.

Blood collection

Blood samples were collected from the fish through caudal peduncle puncture as described by (AQUALEX, 2004). Approximately 60µl of heparin was drawn from EDTA bottle using 2 ml plastic syringe with 22 gauge hypodermic needle (Schmitt *et al.*, 2007). 0.3 - 0.5 ml of blood was collected from each fish and deposited into bottle containing EDTA. The samples were transported in a cold pack to the haematology laboratory at Prof. Umaru Shehu specialist hospital Maiduguri for haematological analysis.

Haematological analysis

The haematological parameters were analyzed using automated haematological analyzer (Model: Sysmex KX-21N, Sysmex Cooperation, Kobe Japan). White blood cell (WBC) count was performed with WBC detector block using Direct Current (DC) detection method. Red blood cell (RBC) and Platelets (PL) were analyzed by Hydrodynamic focusing DC detection methods. Haemoglobin (Hb) levels were analyzed by Non-cyanide Sodium Lauryl Sulphate (SLS) method, while Packed Cell Volume (PCV) was determined using Cumulative pulse height detection (CPHD) method. The blood sample with EDTA was mixed gently and probed with haematological analyzer, and then start bottom was pressed. The haematological parameters were printed out immediately.

Physico-chemical parameters

Water quality parameters such as dissolved oxygen, pH and Temperature during sample collection were collected were recorded using digital EC/TDS/pH kit (model: EC500 Meter SANXIN- China)

Data analysis

Haematological data obtained from all the treatments (cross combinations) were subjected to one way analysis of variance (ANOVA). Differences between the means were determined using Duncan's multiple range tests, Duncan (1955) with SPSS.15 for windows.

RESULTS

Table 1 shows mean blood profiles of juvenile inter-generic hybrid catfish *C. anguillaris* and *H. bidorsalis* and their pure line progenies in North east Nigeria. PCV, Hb, MCHC and platelet (PLT) were higher in pure line *C. anguillaris*. There were no significant differences between PCV, Hb, and PLT values among the entire cross combination. MCHC values from *H. bidorsalis* (34.57 ± 0.33 g/dl) and their maternal hybrids (33.67±0.44) were significantly ($p<0.05$) lower than those recorded in pure *C. anguillaris* (39.03 ± 1.96 g/dl) and their maternal hybrids (35.23 ± 0.61 g/dl). However, MCHC values of pure *H. bidorsalis* juveniles were not significantly ($p>0.05$) higher compared to the reciprocal hybrids. Similarly, MCHC values of pure *C. anguillaris* were significantly the same with to that of their maternal hybrids (*Clariabranchus*).

Table 1: Mean (±SEM) of blood profiles juvenile inter-generic hybrid catfish (*C. anguillaris* and *H. bidorsalis*) and their pure lines progenies in North east Nigeria.

Parameters	Cross combinations			
	C. anguillaris	***Clariabranchus***	***H. bidorsalis***	***Heteroclarias***
PCV (%)	28.65±2.71 ^{a}	25.57±1.12 ^{a}	27.50±1.47^{a}	27.87±1.02 ^{a}
Hb (g/dl)	10.33±0.62 ^{a}	9.00±0.26 ^{a}	9.27±0.58^{a}	9.63±0.28 ^{a}
WBC (x10/µl)	188.98±4.88^{a}	181.53±3.57 ^{a}	192.87±8.29^{a}	193.70±3.26 ^{a}
RBC (x10^3/ µl)	2.42±0.12 ^{a}	2.18±0.10 ^{ab}	1.99±0.14 ^{b}	2.46±0.03 ^{a}
MCV (fl)	117.68±4.83^{a}	117.13±1.76 ^{b}	138.07±1.98^{a}	113.07±2.59 ^{b}
MCH (pg)	42.65±1.99^{b}	41.30±1.15 ^{bc}	46.53±0.70^{a}	39.1±0.61 ^{c}
MCHC (g/dl)	39.03±1.96^{a}	35.23±0.61 ^{ab}	33.67±0.44 ^{b}	34.57±0.33 ^{b}
PLT (x10^3/µl)	19.25±4.78^{a}	17.33±4.81 ^{a}	10.67±8.17 ^{a}	16.67±4.63 ^{a}
LYM (x10^3/µl)	185.65±5.13^{a}	177.97±3.61 ^{a}	187±13.86 ^{a}	190.40±3.43 ^{a}
LYM (%)	98.23±0.22 ^{a}	98.03±0.29 ^{a}	97.37±0.80 ^{a}	98.30±0.12 ^{a}

Means in the same row have having different superscript are significantly difference ($p<0.05$)

Key: PCV= Packed cell volume, Hb = Haemoglobin, WBC = White blood cells, RBC = Red blood cells, MCV = Mean corpuscular volume, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration, PLT = Platelet count, LYM = Lymphocytes.

White blood cell (WBC)

The highest WBC was recorded in hybrid *Heteroclarias* followed by their maternal pure line *H. bidorsalis*, *C. anguillaris* and hybrid *Clariabranchus*. There was no significant difference between the WBC values among the entire cross combination.

RBC counts were higher (2.46±0.03 x10^3/ µl) in *Heteroclarias* followed closely by *C. anguillaris* and the hybrid *Clariabranchus* (2.42±0.12 and 2.18±0.10 x10^3/ µl), while *H. bidorsalis* (1.99±0.14x10^3/ µl) was significantly ($p<0.05$) lower than the other cross combinations. The high RBC values recorded in *Heteroclarias* juveniles were not significantly different from that of *C. anguillaris* and *Clariabranchus*.

Mean Corpuscular volume (MCV

The highest MCV were observed in *H. bidorsalis* (138.07 ± 1.98 fl), followed by *C. anguillaris* (117.68 ± 4.83 fl), *Clariabranchus* (117.13 ± 1.76 fl) and *Heteroclarias* (113.07 ± 2.29 fl). There is no significant difference between MCV values obtained in *H. bidorsalis* compared to that of *C. anguillaris*. Similarly MCV values of *Clariabranchus* and *Heteroclarias* were statistically ($p>0.05$) the same.

Mean cell haemoglobin (MCH)

MCH were observed to be higher in *H. bidorsalis* (46.53 ± 0.70 pg), followed by pure *C. anguillaris* with (42.65 ± 1.99) and *Heteroclarias*. MCH value of *H. bidorsalis* is significantly ($p<0.05$) different from the rest of the cross combinations, while MCH values of *C. anguillaris* and that of their maternal hybrids (*Heteroclarias*) are statistically ($p>0.05$) the same.

Lymphocytes

Lymphocytes values were higher (98.30 ± 0.12 x10^3/µl) in *Heteroclarias*, followed closely by 98.23 ± 0.22 x10^3/µl and 98.03 ± 0.29 x10^3/µl observed in that of pure *H. bidorsalis* and *Clariabranchus* juveniles, respectively. There were no significant difference ($p>0.05$) among the LYM values of the entire cross combination. Percentage LYM were also high in hybrid *Heteroclarias*. Significant difference did not exist between all the percentages LYM for all the treatments.

Water quality parameters

Temperature in the ponds where the fish sampled collected were between 29.43 ± 0.90 ^oC to 30.4 ± 0.12 ^oC, pH 7.49 ± 0.35 to 7.85 ± 0.30 while dissolved oxygen was between 4.36 ± 0.42 to 5.14 ± 0.82 mg/L (Table 2).

Table 2: Water quality parameters of the pond during experimental fish collection

Fish Species	Temperature	pH	DO mg/L^{-1}
C. anguillaris	30.4 ± 0.12	7.74 ± 0.11	4.50 ± 0.38
Clariasbranchus	30.1 ± 0.00	7.85 ± 0.30	5.02 ± 0.64
H. bidorsalis	29.43 ± 0.90	7.49 ± 0.35	4.36 ± 0.42
Heteroclarias	30.13 ± 0.29	7.67 ± 0.41	5.14 ± 0.82

DISCUSSION

Water quality parameters recorded in the ponds where the experimental fish were collected were within the ranger for fish culture recommended by Boyd (1981).

Packed cell volume

PCV ranges of juvenile hybrids and pure line African catfish (*C. anguillaris* x *H. bidorsalis*) recorded in this study fall within normal values of 20 to 35 % (Erondu *et al.*, 1993) and 22 – 40 % (Ram-Bhasker and Srinvasa-Rao 1989). PCV values of hybrids recorded in this study were lower than that of *Heteroclaria* juvenile (38.40 %) (Kori-Sia-kpere and Ubogu 2008) and that of control juvenile *C. gariepinus* (39.00 %) (Ogunji *et al.*, 2005). The difference in the blood profiles could be due to variation in environmental factors (Fernades and Mazon 2003), that haematological characteristics are closely related to the response of the animal to its environment. Accordingly, the environment where the fish lives could exert some influence on the haematological characteristics of that species (Kori-Siakpere, 1985). However, the PCV values recorded in this study are higher than those recorded by Ochang *et al.*, (2007) for control juveniles of *C. gariepinus* (22.00 %) during trails on growth performance, body composition, haematology and product quality of the African catfish (*C. gariepinus*) fed diets with palm oil.

Haemoglobin concentration

Hb reported in this study for the entire cross combinations (9.00 – 10.33 g/dL) is approximate to 10.63 g/dL reported by Osuigwe e*t al*., (2005) for controlled juvenile hybrid between *H. longifilis* x *C. gariepinus***,** but higher than (15.31g/dL) documented by Kori-Siakpere and Ubogu (2008) for juvenile hybrid as well as 13.00g/dL recorded from *C. gariepinus* juvenile by (Ogunji *et al*., 2007). Similarly, 27.00 g/dL reported by Sunmonu and Oloyede (2008) varied with the result of this study. This variation may be due to difference in the species and environment where the fish lived. The high level of Hb recorded in pure line juveniles

C. anguillaris indicates that this trait of high Hb might have been inherited from the paternal parents by the hybrids *Heteroclarias*.

White blood cell

The high WBC recorded in the entire cross combinations higher than those reported by most author viz: WBC (181.53±3.57 to 193.70±3.26) recorded in this study are higher than those reported by most authors viz: 18.8 Ogunji *et al*., (2007), 37.78 for wild adult *C.gariepinus* Gabriel *et al*. (2004), 8.42 for juvenile *Heteroclaria* Osuigwe *et al*, (2005) 49.73 wild *Clariabranchus* (*C.gariepinus x H.bidorsalis*) 16.51 adult *C.anguillaris*, 9.04 *C.macromystax* Bunmi (2010) in North east Nigeria**..**

in this study show significant difference from what most authors reported. However, the high WBC (193.70x10^3/µL) observed in ♀Hb x ♂Ca indicates stronger immune system toward invasion by foreign organism, prevent infection and at least transport and distribute antibodies in immune response as suggested by Sunmonu and Oloyede (2008). The increase in the WBC may be due to environmental stresses of the semi-arid zone. Stress-mediated condition may trigger the release of more white blood cells into the blood stream. myDr. (2009), reported that an abnormal high white cell counts can indicates many possible medical conditions. This may suggest that the *H. bidorsalis* and their maternal hybrids may be affected by the harsh weather conditions of North east region during intensive aquaculture.

Red blood cell

Mean RBC values obtained *Heteroclarias*, *C. anguillaris* and *Clariabranchus* (2.46, 242, and 2.18 x 10^3/µL, respectively) in this study are higher (1.63 x10^{12}) than reported by Kori-Sia-kpere and Ubogu (2008), 1.43 x10^{12}, Osuigwe *et al*., (2005) and 1.77 x 10^6mm^3 (Maheswaran *et al*., 2008). Higher RBC indicated that the level of oxygen that will be transported to the tissue and the level of carbon dioxide returned to the lungs will also increase, thus efficient oxygen supply, survival, and resistance to environmental conditions. However, Ogueji *et al*, (2005) observed as high as 1.80 x 10^6mm^3 RBC from juvenile *C. gariepinus* in Zaria, Nigeria, while Sunmonu and Oloyede (2008), reported 298.50 x 10^{12}/µL from juvenile *C. gariepinus* also from in Ilorin Nigeria. These values disagree with the results obtained in this study. The variation in the RBC could be due variation in the ecological conditions as suggested by Orun *et al*. (2003) that blood parameters are influenced by water temperature and oxygen concentration.

Mean corpuscular volume

MCV is an estimate of the volume of red blood cells. The mean MCV values observed in this study are lower than 240.18 fl recorded for juvenile hybrid African catfish reported by Kori-Sia-Kpere and Ubogu (2008) for juveniles *Heteroclarias* and 200.93 fl for *C. gariepinus* fingerlings (Gbore *et al*., 2006). It is higher than 96.62 fl for *C. gariepinus* fingerlings (Ochang *et al*, 2007). However, the high MCV may be due the high concentration of haemoglobin in the red blood cell.

Mean cell haemoglobin

The MCH values observed in this study are higher than earlier reports 24.24 pg *C. gariepinus* juveniles Omitoyin, (2006), and 33.10 pg Ochang *et al*, (2007), respectively. Kore-Siapere and Ubogu (2008) reported higher MCH for juvenile *Heteroclarias* while Gbore *et al*, (2006) reported 51.50 pg which contrary to what was obtained this study. This explains that the red cell enlargement due probably to nutritional deficiencyof folic acid or Vitamin B12. In another word higher MCH indicates good volume of haemoglobin which indicates good oxygen transportation in the blood stream for healthy well being of the fish.

Mean cell haemoglobin concentration

MCHC values recorded among the entire cross combination are within the range recommended by Ram-Bhasker and Srinvasa-Rao (1989) for healthy fish, except for ♂Ca, ♀Ca which is slightly higher than the rest of the fish species. MCHC for *Clariabranchus* (35.27g/dL) very close to (35.47 g/dL/) for *Heteroclarias* juvenile reported by Kore-Siapere and Ubogu (2008).

Platelet count

Platelet values recorded in this study are lower than 132 x 10^3/µl for juvenile *C. gariepinus* reported by Sunmonu and Oloyede (2008) and 175.92 x 10^3/µl for *Sarotherodon melanotheron* adult (Akinrotimi *et al.*, 2007). The high platelet values observed in *C. anguillaris* and their maternal hybrid indicates that the fish species are likely to withstand and heal from bruises that might have been acquired during fighting and prevention of excessive bleeding via enactment of rapid clotting at injury site.

Lymphocytes (LYM)

The higher lymphocytes observed in hybrid *Heteroclarias* and pure *C. anguillaris* suggested immunity for these two compared to the other cross combinations though there was no significant difference between the lymphocytes values among the entire cross combinations. Hence have the potentials of thriving well in the harsh conditions of North east Nigeria. The lymphocytes in this study are higher than 33.00% for juvenile *C. gariepinus* Adeyemi (2005) and 82.8% for juvenile *C. gariepinus* reported by (Yaji *et al*., 2007).

Conclusion

The high PCV, Hb, MCHC and PLT observed in pure line *Clarias anguillaris* concluded that, they are hardier than both their maternal and paternal hybrids. However, since most of the blood profiles of the entire cross combinations fall with the desired blood profiles of healthy fish this indicates that fish hybrids can withstand the culture conditions in region in comparism to their pure parental progenies.

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